

# The Effect of Fruit Maturity and Storage Duration on Friction Discolouration of ‘Packham’s Triumph’ and ‘Doyenne du Comice’ Pears

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**Keywords:** *Pyrus communis*, impact recording device, electronic apple, skin browning, belt burns, friction bruises, fruit quality.

## Abstract

Friction discolouration (FD) is causing the South African pear industry multi-million rand losses due to blemished fruit being rejected for the export market and being sold locally. The occurrence of FD was studied over two seasons using ‘Packham’s Triumph’ and ‘Doyenne du Comice’ (*Pyrus communis*) fruit. The influence of fruit maturity and storage duration were investigated by harvesting over three maturities and storing fruit for up to three months. FD was induced using a modified laboratory shaker that was shown to closely mimic pack line damage. Polyphenol oxidase (PPO) activity and total phenolics (TP) content were also evaluated. ‘Doyenne du Comice’ was more prone to FD than ‘Packham’s Triumph’. Harvest maturity significantly influenced FD susceptibility, with the middle picking maturity generally giving higher FD ratings. Increasing storage duration generally increased FD ratings, although not consistently. PPO activity was not influenced by harvest maturity, but was influenced by storage duration. In ‘Packham’s Triumph’, the TP content was not affected by harvest maturity or storage duration, whereas both these factors significantly influenced TP content in ‘Doyenne du Comice’. Susceptibility to development of FD symptoms is not easily linked to either PPO activity or TP content, and seasonal differences in susceptibility make prediction of possible levels of FD very difficult.

## INTRODUCTION

Friction discolouration (FD) is a well known and serious postharvest problem in the pear industry (Wang and Mellenthin, 1973; Mellenthin and Wang, 1974). The reduction of visual quality is one of the foremost causes for consumer discontent (Raese, 1989). FD is also referred to as skin browning, abrasion marks, belt burns and friction bruises (Smith, 1946). This disorder is characterised by diffuse brown skin discolourations, especially at high points on irregular fruit surfaces (Meheriuk et al., 1994). Such discolourations are induced by a number of mechanical injuries that fruit are subjected to during harvest, packing, transportation and marketing (Mitcham et al., 2001; Feng et al., 2004), followed by biochemical reactions that lead to browning (Jiménez-Atiénzar et al., 2004).

The effect of fruit maturity on susceptibility to skin discolouration has been studied in ‘Bartlett’ (Mitcham et al., 2001), ‘Doyenne du Comice’ (Amarante et al., 2001a) and an array of cultivars by both Kvåle (1979, 1988) and Amiot et al. (1995). It has been reported that the timely harvesting of the fruit might influence the degree to which this disorder is experienced. It is commonly accepted that both bruising and frictional forces give rise to the skin browning, at epidermal level (Mitcham et al., 2001).

Storage duration as a controllable component in the post harvest chain has been studied by a number of researchers (Mellenthin and Wang, 1974; Kvåle, 1988; Spanos and Wrolstad, 1990; Amiot et al., 1995; Mitcham et al., 2001).

Polyphenol oxidase (PPO) enzyme activity is known to influence the extent and degree of browning of pear peel (Gauillard and Richard-Forget, 1997). Sufficient and

acceptable substrate, among others, is needed for enzymatic browning to take place (Goupy et al., 1995). These substrates come in an array of chemical compounds called phenols (Harbone and Simmonds, 1964). The availability of these compounds within the pear peel is fundamental in these biochemical reactions (Amiot et al., 1995).

This study was conducted to evaluate the susceptibility of 'Packham's Triumph' and 'Doyenne du Comice' to FD in terms of fruit maturity and storage duration. Additionally, the influence of maturity and storage duration on PPO activity and total phenolics (TP) content of the peel were also evaluated.

## **MATERIALS AND METHODS**

Research was conducted in the 2003 and 2004 seasons with 'Packham's Triumph' and 'Doyenne du Comice' fruit from Grabouw and Ceres, Western Cape, South Africa.

### **Determination of Friction Discolouration Susceptibility**

An electronic impact-recording device (IRD400, Techmark, Inc., USA) was used to determine the level of impacts encountered on a commercial pear packing line. These data were used as reference during laboratory simulation, to ensure that treatments were representative of the commercial situation. A laboratory shaker (RO 30, Gerhardt, Bonn) was modified to simulate the packing practices. The IRD was used to identify the shaker velocities (in revolutions per minute, rpm) that yielded similar Max G values as those measured on the packing line. A corrugated cardboard box (280 x 370 x 80 mm, length x width x height) was lined with smooth transport belt, as used in packhouses, wherein the sampled fruit was placed. These fruit were then subjected to the specific treatment for 2 minutes and the FD was calculated 24 hours later by means of the skin-browning index (SBI) (Mitcham et al., 2001).

Skin browning index =  $[(Ax1 + Bx2 + Cx3 + Dx4 + Ex5) \times 0.75 + (Fx0.25)] / \text{Total \# fruit}$

A = # pears with < 1 % brown area

B = # pears with 1-2 % brown area

C = # pears with 3-5 % brown area

D = # pears with 6-10 % brown area

E = # pears with > 10 % brown area

F = total value of brown colour intensity for all pears evaluated\*

\*Colour intensity was subjectively recorded on a 1-to-5 scale, with 1 = low intensity and 5 = high intensity.

Each repetition consisted of 5 fruit. In 2003, velocities of 75, 85, 95 and 105 rpm were used, and in 2004 only 105 rpm, which was found to relate best to average pack line conditions. Using this methodology, the influence of fruit maturity and storage duration on FD susceptibility was assessed as described below.

### **Fruit Maturity**

Each cultivar was harvested on three different dates within the commercial picking window. 'Doyenne du Comice': 15, 22 and 29 January 2003, and 20, 25 and 30 January 2004. 'Packham's Triumph': 29 January, 5 and 12 February 2003 and 30 January, 6 and 13 February 2004. Fruit firmness was determined on opposite, peeled sides of representative fruit using a penetrometer (Southtrade, FT 327, Italy) fitted with an 8 mm tip. Undamaged fruit skin samples were taken from the equatorial region from each of the fruit in a repetition immediately following determination of the SBI. Peel tissue was frozen in liquid nitrogen and stored at -80°C until analysis of TP content and polyphenol oxidase PPO activity.

### **Fruit Storage**

Fruit were evaluated for FD susceptibility immediately following harvest and after 1, 2 or 3 months of storage in polyethylene-lined telescopic cartons held at 0°C under regular atmosphere conditions. Stored fruit were first treated with a 2% iprodione

(Aventis CropScience) fungicide to prevent development of decay. Once again, peel tissue was taken for analysis of TP content and PPO activity.

### **Enzyme (PPO) Extraction**

The PPO extraction and analysis procedure was a modification of the methods used by Barrett et al. (1991) and Mitcham et al. (2001). All the steps, where possible, were carried out on ice. One gram of finely ground pear peel was added to 1 g polyvinylpyrrolidone (PVPP) and 9 ml of chilled extraction buffer (0.05 M phosphate, 1 M KCl, pH = 7). This mixture was stirred for 10 minutes at 8°C before filtering through one layer of cheesecloth. The filtrate was centrifuged (14 000 x g) for 30 minutes at 4°C. The supernatant was again filtered, using Whatmann #4 filter paper (Whatmann International Limited, Kent, England).

### **Determination of PPO Activity**

The sample blank contained 1.96 ml of the reaction buffer (0.2 M Phosphate, 0.1 M Citrate, pH = 6.5) as well as 0.44 ml of a catechol solution (0.5 M catechol in a 10 fold dilution of the reaction buffer). Each sample contained 0.2 ml extract, 1.76 ml reaction buffer and 0.44 ml of the standard catechol solution. Before the cuvette was placed inside the spectrophotometer it was thoroughly shaken for 3 seconds. The change in absorbance at 420 nm was followed over time using a Varian Cary 3C spectrophotometer (Varian Analytical Instruments, CA). The activity of the enzyme (PPO) was calculated using the initial gradient (first 24 seconds) of the curve that was obtained. PPO activity is presented as the change in absorbance at 420 nm per gram fresh pear peel per minute.

### **Extraction and Measurement of Total Phenolics (TP)**

The phenolic compounds were extracted, partially purified and the total phenolic content was determined by means of a Folin-Ciocalteu (FCR) method, based on the methods used by Kim et al. (2003) and Mitcham et al. (2001). Freeze-dried peel samples were ground to powder under liquid nitrogen with a mortar and pestle. To each sample of 1 g of powdered fruit skin, 10 ml of 80% ethanol was added before stirring for 2 hours at 8°C. The extract was filtered using Whatmann #2 filter paper.

A series of gallic acid solutions (30, 60, 100, 200, 300, 400, 500 mg/l) was prepared to serve as calibration standards. To each sample, consisting of 0.4 ml extract, 4 ml ddH<sub>2</sub>O and 0.4 ml FC reagent was added. This was then shaken and left for 5 minutes. After this period, 4 ml Na<sub>2</sub>CO<sub>3</sub> (7%; m/v) and 1.2 ml ddH<sub>2</sub>O was added. After a 90 minute incubation period, the absorbance was measured at 750 nm against the standard curve using a Varian Cary 3C spectrophotometer (Varian Analytical Instruments, CA). Total phenolic content is expressed in mg gallic acid per gram dry pear peel.

### **Statistical Analysis**

Statistical Analysis Systems (SAS), Enterprise Guide was used to determine the analysis of variance (ANOVA) and LSD values with a 5 % significance level. A complete randomised design was used for the data set. In the cases where interactions were present, contrasts were examined. All the experiments were done in 6 replicates.

## **RESULTS**

### **Friction Discolouration Simulation**

Increasing the velocity of the shaker between 75 and 105 rpm led to higher impact values (Max G values) recorded by the IRD (Figure 1). Max G values were also related to the degree of FD shown by the fruit (data not shown), hence it was concluded that the laboratory-scale methodology employed gave a true reflection of the damage experienced during commercial fruit packing.

### **Fruit Maturity**

Fruit firmness declined significantly for both cultivars in both seasons as maturity progressed from Harvest 1 to Harvest 3 (Table 1). Firmness readings were within the commercial maturity spectrum and fruit could therefore be considered as representative of the commercial crop.

Fruit maturity significantly influenced FD susceptibility in both cultivars and both seasons (Table 2). For 'Packham's Triumph', fruit from Harvest 2 were significantly more prone to FD than fruit from Harvest 1 or 3. This was also the case for 'Doyenne du Comice' in 2004, but in 2003 the Harvest 2 fruit were less susceptible to FD, although FD values were much higher in 2003 than in 2004. 'Doyenne du Comice' showed a greater degree of discolouration than 'Packham's Triumph' throughout all the simulations.

### **Storage Duration**

In 2003, 'Packham's Triumph' showed an increasing susceptibility to FD as storage interval increased, whereas FD susceptibility peaked after 2 months storage in 2004 (Table 2). In 'Doyenne du Comice', FD susceptibility was high in 2003 and peaked after 3 months cold storage, whereas it tended to decrease with storage duration in 2004, reaching a low point after 2 months storage (Table 2).

### **PPO Activity**

PPO activity was not influenced by maturity in either cultivar, but was significantly influenced by storage duration (Table 3). PPO activity appeared to be slightly greater in 'Packham's Triumph' than in 'Doyenne du Comice'.

### **Total Phenolic Content**

The TP content of 'Packham's Triumph' was not influenced by harvest maturity or storage duration (Table 3). However, both these variables significantly influenced the TP content of 'Doyenne du Comice' (Table 3). TP levels were typically higher in 'Doyenne du Comice' than in 'Packham's Triumph'.

## **DISCUSSION AND CONCLUSION**

### **Friction Discolouration Simulation**

The degree of FD was higher in 2003 than in 2004 (Table 2). The reason for this is unknown, although seasonal differences in occurrence of the disorder are commonly experienced by the pear industry. 'Doyenne du Comice' is known as a very sensitive cultivar, as is demonstrated by the higher FD ratings than for 'Packham's Triumph', especially in 2003.

### **Fruit Maturity**

Fruit firmness measurements indicated that comparable fruit were used during both years (Table 1). The effect of harvesting date proved to be substantial during both years in terms of FD occurrence in 'Packham's Triumph' and 'Doyenne du Comice' (Table 2). In both seasons, the second harvest date yielded the highest SBI score in the case of 'Packham's Triumph'. This harvest maturity yielded the lowest SBI score for 'Doyenne du Comice' in 2003 but highest in 2004. Mitcham et al. (2001) also found that skin browning closely correlated with fruit firmness, where firmer fruit showed less browning. This, however, is in contrast with studies by Kvåle (1979), where more mature fruit, which were less firm, proved to be the least susceptible to discolouration. Such discrepancies may be due to the method used to induce the damage.

### **Fruit Storage Duration**

Increasing storage duration generally led to an increase in FD susceptibility, especially in 2003 (Table 2). From previous studies it appears that susceptibility to FD tends to increase as storage duration is prolonged (Mitcham et al., 2001). This is largely

attributed to the accumulation of phenolic compounds present in the pear peel (Wang and Mellenthin, 1973; Mellenthin and Wang, 1974; Meheriuk et al., 1982; Kvåle, 1988), the decrease in fruit firmness (Kvåle, 1988; Amiot et al., 1995; Mitcham et al., 2001) and fruit desiccation (Amarante et al., 2001a). Moisture loss renders the fruit less turgid, which influences the cell membrane integrity negatively and makes the cell more susceptible to frictional damage.

### **PPO Activity**

Harvest date did not have any significant influence on the activity of PPO (Table 3). However, storage duration did prove to have a significant effect, albeit not consistently for both cultivars. The activity of PPO extracted from 'Packham's Triumph' was higher than what was found for 'Doyenne du Comice' (Table 3), even though SBI values were lower (Table 2). This may be explained by the fact that the enzyme only lowers the activation energy and thereby catalyses the reaction without taking part in the reaction itself. It is also possible that the abundance of PPO is higher in 'Packham's Triumph' than in 'Doyenne du Comice', as our data are not expressed relative to protein abundance.

### **Total Phenolic Content**

As previously reported (Amiot et al., 1995), it was found that TP content of 'Doyenne du Comice' increased slightly with an increase in storage duration (Table 3). This was, however, not found to be the case with 'Packham's Triumph'. It appears as if PPO activity and TP content are roughly inversely proportional to each other. Often, when PPO activity was low, a relatively high TP content was recorded. This emphasises the fact that, when the oxidative process is suppressed by whichever means, the TP content should increase due to the slower conversion rate to quinones. In the case of 'Packham's Triumph' (Tables 2 and 3) it appears as if high SBI values were obtained when the PPO activity was relatively high, rather than when the TP content was high. However, this relationship is not as clear in the case of 'Doyenne du Comice' (Tables 2 and 3). Given the rapid development of FD symptoms, especially in the case of 'Doyenne du Comice', it seems as if neither PPO nor TP are limiting factors in FD development. The different rates of browning symptom development between the two cultivars may be due to anatomical differences in the dermal structures. It has been shown, for example, that dermal permeation to oxygen is higher in 'Doyenne du Comice' than in 'Packham's Triumph' (Amarante et al., 2001b). It is, therefore, conceivable that 'Doyenne du Comice' browns faster due to a greater availability of oxygen required for the functioning of PPO.

### **ACKNOWLEDGEMENTS**

The financial assistance of TruCape (Pty) Ltd. is gratefully acknowledged, as is the assistance of packhouse personnel from Kromco (Grabouw) and Ceres Fruit Growers (Ceres). PPO and TP analyses were conducted with the very capable assistance of Elizabeth Rohwer.

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## Tables

Table 1. Mean fruit firmness of ‘Packham’s Triumph’ and ‘Doyenne du Comice’ as influenced by sequential harvest dates in 2003 and 2004.

Season	Treatment	Firmness (kg) <sup>1</sup>	
		‘Packham’s Triumph’	‘Doyenne du Comice’
2003	Harvest 1	8.19 a	7.66 a
	Harvest 2	7.64 b	6.90 b
	Harvest 3	6.74 c	5.81 c
	LSD	0.4925	0.3116
	Pr>f	<0.0001	<0.0001
2004	Harvest 1	8.10 a	6.98 a
	Harvest 2	7.21 b	6.06 b
	Harvest 3	6.53 b	5.91 b
	LSD	0.6896	0.4145
	Pr>f	0.0003	<0.0001

<sup>1</sup>Means separated within seasons and cultivars using least significant differences (0.05).

Table 2. Effect of fruit harvest maturity (harvest date) and storage duration on friction discolouration susceptibility expressed as skin browning index (SBI) values for ‘Packham’s Triumph’ and ‘Doyenne du Comice’ pears.

Season	Treatment	Skin browning index (SBI) <sup>1</sup>	
		‘Packham’s Triumph’	‘Doyenne du Comice’
2003	Harvest 1	0.46 c	2.54 a
	Harvest 2	1.25 a	1.96 b
	Harvest 3	0.54 b	2.38 a
	LSD	0.0695	0.1669
	Pr>f	<0.0001	<0.0001
	At harvest	0.48 c	2.18 b
	After 1 month storage	0.66 b	2.16 b
	After 2 months storage	0.73 b	2.29 b
	After 3 months storage	1.13 a	2.53 a
	LSD	0.0803	0.1927
	Pr>f	<0.0001	0.0010
2004	Harvest 1	0.18 b	0.43 b
	Harvest 2	0.35 a	0.59 a
	Harvest 3	0.21 b	0.36 b
	LSD	0.076	0.0843
	Pr>f	<0.0001	<0.0001
	At harvest	0.10 c	0.73 a
	After 1 month storage	0.23 b	0.41 b
	After 2 months storage	0.41 a	0.28 c
	After 3 months storage	0.24 b	0.42 b
	LSD	0.0877	0.0974
	Pr>f	<0.0001	<0.0001

<sup>1</sup>Means within main effects and cultivars are separated using least significant differences (0.05).

Table 3. Polyphenol oxidase (PPO) activity and total phenolic (TP) concentration of 'Packham's Triumph' and 'Doyenne du Comice' pear peel in 2004.

Treatment	'Packham's Triumph'		'Doyenne du Comice'	
	PPO (change in A <sub>420</sub> /g/minute) <sup>1</sup>	TP (mg gallic acid equivalents/g)	PPO (change in A <sub>420</sub> /g/minute)	TP (mg gallic acid equivalents/g)
Harvest 1	12.3 a	61.0 a	9.12 a	99.0 a
Harvest 2	13.0 a	59.2 a	9.79 a	89.6 b
Harvest 3	12.2 a	60.8 a	9.75 a	88.8 b
LSD	1.2776	3.2027	1.0188	5.227
Pr>f	0.4247	0.4911	0.3488	0.0003
At harvest	12.2 b	62.5 a	10.4 a	91.1 a
After 1 month storage	12.9 b	60.4 a	9.70 a	90.3 a
After 2 months storage	14.4 a	59.0 a	10.3 a	90.4 a
After 3 months storage	10.5 c	59.5 a	7.80 b	98.0 b
LSD	1.4752	3.6982	1.1764	6.0356
Pr>f	<0.0001	0.2452	<0.0001	0.0341

<sup>1</sup>Means within main effects and cultivars are separated using least significant differences (0.05).

## Figures

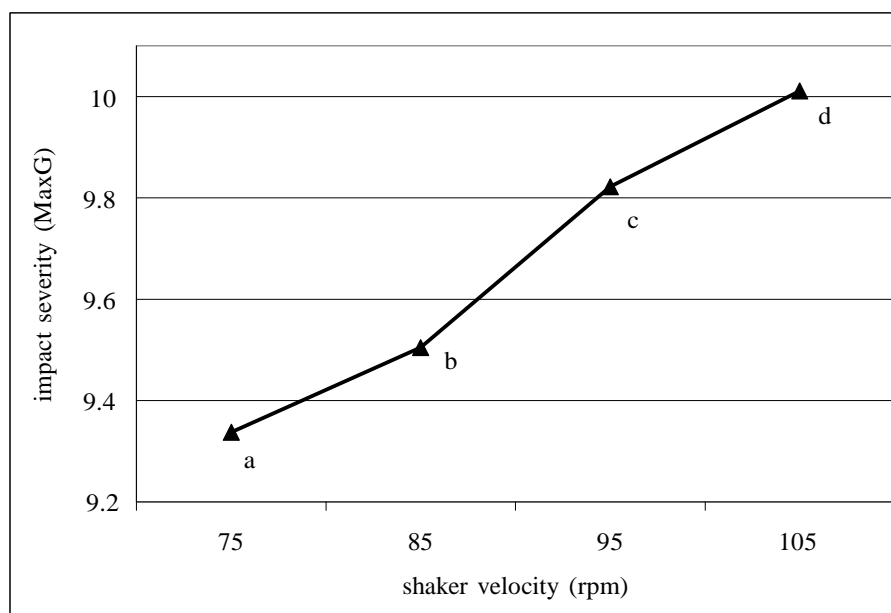


Fig. 1. Relationship between the shaker velocity and impact severity as recorded with an electronic impact recording device.